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TITLE: Novel Purine Derivatives Having, In Particular, Antiproliferative Properties and Biological Uses Thereof.

Abstract: 2-, 6- and 9- subsituted purines derivatives, particularly 2-(1-R hydroxymethylpropylamino)-6-benzylamino-9-isopropylpurine, having, in particular, antiproliferative properties, and suitable for use as drugs and weedkilers, are disclosed.

Novel Purine Derivatives having, in particular, anti-proliferative properties and biological applications thereof.

This invention is related to novel purine derivatives having anti-proliferative properties and the biological applications thereof. More particularly, it is related to purine derivatives that have an inhibiting effect on protein-kinases cyclin-dependent, or cdk.

The study of the molecular mechanisms that control the cell cycle has shown the regulatory role of the cdk. These proteins have at least two subunits, one catalytical (cdc2 is the prototype) and one regulator (cyclin). Eight cdk have been described, cdk1 (=cdc2), cdk2-cdk8.

Except for cdk3, for which no associated cyclin has been described, these cdk are regulated by transient association with one member of the cyclins family: cyclin A (cdc2, cdk2), cyclin B1-B3 (cdc2), cyclin C (cdk8), cyclin D1-D3 (cdk2-cdk4-cdk5-cdk6), cyclin E (cdk2), cyclin H (cdk7).

Each of these complexes is involved in a phase of the cell cycle. The activity of the cdks is regulated by post-translational modification, by transient associations with other proteins, and by modification of their intracellular location. The compounds that regulate the activity of the cdks comprise activators (cyclins, cdk7/cyclin H, phosphatases cdc25), the $p9^{cks}$ and p15 $^{cdk\text{-}BP}$ subunits, and inhibiting proteins (p16 ^{INK4A}, $p15^{INK4B}$, p21 ^{cip1}, p18, $P27^{kip1}$).

In parallel to fundamental research studies on the regulation mechanisms of the cell cycle, numerous studies have demonstrated the importance of the deregulation of cyclin-dependent kinases in the development of human tumors. Thus, the over-expression of cyclins D and E in many tumors, the over-expression of cdc2, the oncogenic properties of cyclins D and A, the abnormal timing in the expression of cyclin-dependent kinases, and the major deregulation of proteic inhibitors (mutations, deletions) have been observed.

The cell cycle regulators are subject to many clinical studies (utilization as markers for the treatment).

These results strongly foster studies aiming to understand the regulation mechanisms of the cell cycle. They also promote the search, by screening, for molecules inhibiting the cyclin-dependent kinases.

Several kinases inhibitors have been described , such as butyrolactone, flavopiridol and 2-(2-hydroxyethylamino)-6-benzylamino-9-methylpurine, also called olomucin. Studies related to olomucin are reported

by Vesely and al. in the article referenced (1) in the bibliographical list provided at the end of the description.

This cdc2 inhibitor, is very efficient (IC_{50}= 7μM) and selective (over 35 kinases have been tested). Its formula is:

[see original]

The inventors' studies in this domain led them to synthesize new and especially interesting molecules which inhibit cdc2 at low doses while retaining the enzymatic specificity of the olomucin.

The invention is then directed to novel purine derivatives having, in particular, anti-proliferative properties.

This invention is also directed to a method for synthesizing these derivatives at the industrial scale.

This invention is also directed to the application of these derivatives as therapeutic compounds and as weedkillers.

The purine derivatives of this invention are characterized by the formula I :

[formula page 3]

in which:

- R2, R6 and R9, identical or different from one another, represent an halogen atom, a radical R-NH-, R-NH-NH, H2N-R'-NH- or R-NH-R'-NH-, in which R can be a straight or branched, saturated or unsaturated alkyl radical, an aryl or cycloalkyl radical, or an heterocycle, R' is a linear or branched, saturated or unsaturated alkylen or an arylen or cycloalkoylen, and R and R' counting each 1 to 8 carbon atoms and being potentially substituted with one or several groups -OH, halogen, amino or alkyl,
- R2 can be a heterocycle potentially bearing a linear or branched, saturated or unsaturated alkyl radical, or a heterocycle potentially substituted by one or several -OH, halogen, amino or alkyl groups
-R9 can be a linear or branched, saturated or unsaturated alkyl radical, an aryl radical or a cycloalkyl radical
- R2 and R9 can also represent a Hydrogen atom, except in derivatives in which the aforementioned substitutives have the following significations:

R6 and R9, a benzylamino and methyl group

R2 and R6, a 2-hydroxyehtylamino and benzylamino group

R2, R6, and R9 , an amino, benzylamino and methyl group, or chloro, amino and methyl group, or chloro, benzylamino and methyle group, or chloro, 3-hydroxybenzylamino and methyl group, or chloro,

5-hydroxypentylamino and methyl group, or 2-hydroxyethylamino, benzylamino and isopropyl group, or 2-hydroxyethylamino, amino and methyl group, or 2-hydroxyethylamino, isopentenyl and methyl group, or 2-hydroxyethylamino, isopentenylamino and methyl group, or 2-hydroxyethylamino, benzylamino and methyl group, or 2-hydroxyethylamino, benzylamino and 2-hydroxyethyl group, or 2-hydroxyethylamino, benzylamino and isopropyl group, or 2-hydroxyethylamino, (3-hydroxybenzyl)amino and methyl group, or 2-hydroxyethylamino, (3-hydroxybenzyl)amino and isopropyl group, or 2-hydroxyisobutylamino, 6-benzylamino and methyl group, 2-hydroxyethylamino, isopentenylamino and isopropyl group, or (2- hydroxyethyl)amino, (4-methoxybenzyl)amino and isopropylamino.

The purin derivatives of the invention are moreover characterized in that they have an iC_{50} inferior to or equal to about 5 μM for cdc2/cyclinB.

The derivatives that are excluded from the invention, and mentioned hereinabove, are described in reference (1).

The derivatives of the invention are in general protein-kinases inhibitors of great interest.

The halogen atom is preferably chosen among Chlorin, Brome or Fluor. The alkyl radical is chosen among the methyl, ethyl, propyl, isopropyl, butyl and isobutyl, pentyl, hexyl and heptyl groups. The alkoylen radical is chosen among the methylen, ethylen, propylen, isopropylen, butylen, isobutylen, penten or isopenten groups. The aryl radical is a benzylen group; the cycloalkyl is a cycohexyl; the arylen radical is a benzylen group; the cycloalkoylen radical is a cyclohexylen group; the heterocycle is a nitrogen and/or oxygen heterocycle such as an imidazol, oxadiazol, pyridin, pyridazin or pyrimidin or else, a pyrrolidin.

According to one provision of the invention, the R2 radical is chosen among those that are able to bind within a cdk2/ATP complex, to a region of the ATP-binding domain occupied by the ribose residue. This radical is advantageously chosen among a chlorin atom, an amino, methylamino, ethylamino, n-heptylamino, aminoehtylamino, aminopropylamino, dimethyl aminoethylamino, hydroxyethylamino, hydroxy-propylamino, hydroxyisobutlyamio, hydroxypentylamino, dimethylhydrazino or hydroxymethyl-propylamino, [(2R)-2-hydroxymethyl-pyrrolidin-1-yl], N- benzyl-aminoethanol, (R,S)-amino-hexanol, (S)-amino-2-phenylethanol, (R)-amino-2-phenylethanol, (R)-amino-3-phenylpropanol, (R,S)-amino-pentanol, (R)-amino-propanol, (S)-amino-propanol, (R)-N-pyrrolidin methanol group.

Particularly preferred derivatives comprise as a R2 group, a hydroxypropylamino radical.

According to another provision of the invention, R6 is chosen among an amino, isopentenylamino, hydroxypentylamino, 4-hydroxy-3-methyl-trans-2-butenylamino, benzylamino, hydroxybenzylamino, hydroxyethylbenzylamino, cyclohexylmethylamino, isopenten, benzylamino or (3-iodo)-benzylamino group.

Preferably, R6 comprises an hydrophobic residue such as benzyl, hydroxybenzyl, or isopentenyl.

Preferably, R2 is chosen among the [1-D,L-hydroxymethylpropylamino], [(2R) -2-hydroxymethyl-pyrrolidin-1-yl], or [(R)-N-pyrrolidin-methanol] and R6 is a benzylamino.

According to another provision of the present invention, the R9 substituant is chosen among a hydrogen atom, a methyl, isopropyl or hydroxyethyl radical.

Advantageously, R9 is a hydrophobic group, particularly the isopropyl group.

Preferred purin derivatives of the present invention are chosen among the compounds in which R2, R6, and R9 are such as indicated in table 1:

[see table 1, page 7]

The following derivatives are especially preferred: 2-(1-D,L-hydroxymethylpropylamino)-6-benzylamino-9-isopropylpurin, non-crystalline 6-benzylamino-2-[(2R)-2-hydroxymethyl-pyrrolidin-1-yl]-9-isopropyl-(9H)-purin, 2-(R)-[6-benzylamino-9-isopropyl-(9H)-purin-2-yl]-amino-2-phenylethanol, 2-(R,S)-[6-benzylamino-9-isopropyl-(9H)-purin-2-yl]-amino-pentanol, 2-(R)-[6-benzylamino-9-isopropyl-(9H)-purin-2-yl]-amino-propanol, 2-(S)-[6-benzylamino-9-isopropyl-(9H)-purin-2-yl]-amino-propanol, 2-(R)-(-)-[6-(3-iodo)-benzylamino-9-isopropyl-(9H)-purin-2-yl]-N-pyrrolidin methanol and 2-(R)-(-)-[6-benzylamino-9-cyclopentyl-(9H)-purin-2-yl]-N-pyrrolidin methanol.

The invention is also directed to optical isomers and racemic mixes, if the case arises, the geometric isomers of the derivatives defined hereinabove, particularly the R isomer of the (2-[6-benzylamino-9-isopropyl-(9H)-purin-2-yl]-amino-2-phenylethanol and of the 2-[6-benzylamino-9-isopropyl-(9H)-purin-2-yl]-amino-propanol.

The derivatives defined hereinabove are obtained by use of the classical organic synthesis methods. A starting purin derivative is used and substituted so that to introduce the groups of interest.

By use , for example, of a purin-2-chloro, 6-benzylamino derivative, it is possible to introduce an alkyl group in position 9 by reaction, for example, with the corresponding alkylhyde.

The reaction with an amino-alcohol will allow next to introduce, in position 2, an alkyl-hydroxyalkylamino group in place of a chloro group.

According to an aspect of great interest, the derivatives of the invention bear kinase inhibiting properties of great specificity. These inhibiting effects are reversible.

As the cdk play a determinant role in the initiation, development and termination of the cell cycle, the molecules inhibiting cdk are likely to limit an undesired cellular proliferation such as in cancer, psoriasis, growth of fungi, parasites (animal, protists), but also of plants (herbicides), and to impact the regulation of neurodegenerative diseases such as the neuronal apoptosis and Alzheimer disease.

The kinases that are more specifically sensitive to the inhibiting effects of these derivatives are the cdc2, the cdks2 and cdks5.

Their inhibition is obtained by use of very low doses of purin derivatives.

Then, an IC_{50} towards cdc2 lower than 50 μM, is generally observed. This IC_{50} is even lower than the IC_{50} of the olomucin yet considered as a powerful inhibitor.

The invention is directed to purin derivatives showing an IC_{50} not exceeding 5 μM, and strikingly the 2-(1-D,L-hydroxymethylpropylamino-)-6-benzylamino-9-isopropylpurin, thereafter called roscovitin, which has an IC_{50} of 0.65 μM, the non-crystalline 6-benzylamino-2-[(2R)-2-hydroxymethyl-pyrrolidin-1-yl]-9-isopropyl-(9H0-purin, the 2-(R)-(-)-[6-(3-iodo)-benzylamino-9-isopropyl-(9H)-purin-2-yl]-N-pyrrolidin methanol.

This derivative, an inhibitor of great efficiency and selectivity towards the cdks, cdc2,cdk2, and cdk5, unexpectedly presents on the other hand similar effects on erk1 and erk2 kinases to those observed with the olomucin. The selectivity is therefore much higher towards cyclin-dependent kinases. This advantage also shared with the other purin derivatives of the invention, allows the elimination of interferences with the transduction signals pathways that are more upstream and that involve the erk1 and erk2 kinases in numerous cellular responses other than the cell division.

The invention is also directed to the purin derivatives complexes with the cdks, and more

especially the crystalline form of the cdk2 complex with the roscovitin.

Studies achieved on the derivatives of the invention have shown, beyond their kinase-specific inhibiting properties, cellular effects and effects on apoptosis of great interest.

Then, at very low concentration, (micromolar for the roscovitin and a significant number of its derivatives), they are able to inhibit the prophase/metaphase transition, as illustrated in experiments performed on starfish oocytes and urchin embryos, and that are reported in the examples.

On acellular extracts of Xenopus, they are able to inhibit both the promoter factor of the M phase and the synthesis of DNA.

Advantageously, these cellular effects are obtained at very low derivatives concentrations.

Miscellaneous studies address the relationship between the cellular cycle and apoptosis. Various pathways lead to cellular apoptosis, some of which depend on kinases, and others do not seem to require these enzymes. It has been shown that apoptosis can be induced at the G1 or G2 stage, and that, as a result of the DNA damage, certain cells stop at the G1 stage and show the induction of a p53 dependent [1]apoptotic pathway.

This pathway is particularly important for the therapy of tumors displaying a loss of active p53.

The derivatives of the invention therefore present a great interest as means to stimulate a p53-dependent apoptosis in cells that are arrested at the G2 stage as a result of DNA damage by agents such as mitoxantrone or cis-platin.

The cdc2 inhibitors of the invention can then augment the therapeutic effects of anti-tumoral agents currently in use.

As inhibitors of cdk 5, the derivatives of the invention can also play a role to reduce the abnormal hyperphosphorylation of tau observed during Alzheimer disease.

An additional interest to these various advantageous properties is that the derivatives of the

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[1] exact translation of French version. Logically, should be: "independent".

invention have no cytotoxicity.

The invention is therefore directed to utilize the properties of these derivatives, particularly their antimitotic and anti-neurodegenerative properties, to elaborate pharmaceutical compositions.

The pharmaceutical compositions of the invention are characterized in that they comprise an efficient amount of at least one purin derivative as defined hereinabove, in association with an inert pharmaceutical vehicle.

The compositions of the invention are especially suited as antimitotic medications, especially for use in cancer chemotherapy, or for the treatment of psoriasis, parasitosis such as those due to protists or fungi, or of Alzheimer disease, or of neuronal apoptosis.

These pharmaceutical compositions can include active principles other than medications. In particular, they can be associated with antimitotic medications such as those using taxol, cis-platin, DNA intercalating agents and others.

The packaging for sale, especially the labellling and "notice d'emploi"[2], and advantageously the packaging, are made accordingly to the therapeutic application.

The pharmaceutical compositions of the invention can be administered under different forms, more especially by oral route or by injection.

For the oral route administration, tablets, capletspills, capsules, drops are particularly used. These formulations advantageously comprise from .1 to 100 mg of active principle per dose, preferably, from 10 to 40 mg.

Other administration forms include intravenous, subcutaneous or intramuscular injectable solutions, elaborated from sterile or sterilizable solutions. They can also be suspensions or emulsions.

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[2] "notice d'emploi" refers to an information slip that is included in each package. This information slip indicates the chemical composition and dosage, the indications, possible secondary effects, possible incompatibilities with other medications, and warnings. It would be similar to a copy of the PDR referring to the drug, withy additional information for the patient.

These injectable forms contain from 1 to 50 mg of active principle per dose, preferably, from 10 to 30 mg.

For example, the dosage that can be utilized in humans corresponds to the following: one or several doses at 10 to 50 mg/day is administered to a patient for the treatment of tumors, or of psoriasis or of parasitosis.

The invention is also directed to weedkiller compositions containing at least one purine derivative, such as defined hereinabove, which can be associated with other phytopharmaceutical agents.

The invention is moreover directed to biological reagents of which the active principles include the abovedefined purin derivatives.

These reagents can be references or standards for use the cell division studies.

Other characteristics and advantages of the invention are reported hereinafter in the examples and accordingly to the figures 1 to 8 in which:

- figure 1 represents the kinetics results in linear conditions from assays concerning the activity of the $p34^{cdc2}$/cyclin B at various roscovitin concentrations,

- figure 2, represents the percentage of starfish germinal vesicle disrupture to the roscovitin concentration

- figure 3 and 4 represent respectively the effects of roscovitin on starfish oocyte maturation and on the *in vivo* tyrosine dephosphorylation of the $p34^{cdc2}$,

- Figure 4 represents the effects of roscovitin on the mitotic cycle of urchin embryos,

- figure 5 represents these embryos stopped at the prophase stage,

- figure 6 represents the effects of roscovitin on the *in vitro* synthesis of DNA and on the MPF activity,

- figure 7 represents the effects of roscovitin on the inhibition of L1210 cell growth and the termination of their cell cycle in G2/M phase, on figure 7A is displayed the growth of L1210 cells after two days of exposure to various concentrations of roscovitin (average $\pm$ standard deviation related to the growth of untreated standard cells0, on figure 7B are represented the averages ($\pm$ standard deviation) of the distribution of the cycle of cells that have been cultivated first during 48 hours in the presence or in the absence of roscovitin 60 µM,

- figure 8 represents the inhibitory effect of roscovitin on the *in vivo* phosphorylation of vimentin at the level of cdc2 specific sites.

Material and Methods

Chemical reagents:

Sodium Ortho-vanadate, 1-methyladenin (1MeAde), EGTA, EDTA, MOPS, β–glycerophosphate, Dithiothreitol (DTT), Natrium fluorin, Nitrophenlphosphate, leupeptin, aprotinin, soy bean trypsin inhibitor, benzamidin, H1 Histon (type III-S), myelin basic protein (MBP), casein, protamin sulfate, isopropyl β–D-thiogalactopyranoside (IPTG), Sepharose 4B activated by CNBr, LB medium, glutathione and glutathione-sepharose beads: all these products are commercialized by Sigma Chemicals.

The purin analogs are generally dissolved such as to obtain 100 mM stock solutions in DMSO. The DMSO final concentration in the reagent mix is lower than 1% (v/v).

The [γ^{32} P]-ATP is an Amersham product.

The GST-retinoblastoma protein is expressed in bacteria and purified on glutathione-Sepharose beads as previously described in (1) and (2).

Buffers:

Homogenization buffer:

β-glycerophosphate 60mM, p-nitrophenyl-phosphate 15 mM, MOPS 25 mM, EGTA 15 mM, $MgCl_2$ 15 mM, DTT 1mM, Natrium vanadate 1mM, NaF 1mM, phenylphosphate 1mM, leupeptin 10μg/ml, aprotinin 10μg/ml, soy bean trypsin inhibitor 10μg/ml, and benzamidin 100μM.

C Buffer:

homogenization buffer composition , but with EGTA 5mM without NaF and without protease inhibitors.

Preparation of the starfish oocyte extracts in M phase:

To obtain large scale oocytes extracts , the gonads are removed from mature *Marthasterias glacialis* and incubated in 10 μM 1-MeAde in natural sea water filtrated on Millipore until laying (ponte). The oocytes are then all in M phase. They are separated from the incubation medium by centrifugation and directly frozen in liquid nitrogen, and maintained at -80deg Celsius (see (1) and (2)).

The oocytes in Phase are homogenized in 2 ml homogenization buffer per gram of oocytes.

After centrifugation during 45 minutes at 100,000 g, the supernatant is removed and directly used for affinity chromatography purification of the $p34^{cdc2}$/cyclin B on $p9^{CKShs1}$-sepharose beads (see (1) and (4)).

Enzymes:

The activities of the kinases are determined at 30 deg Celsius in C buffer, unless contradictory indication. The standard values are subtracted from the data and the activities are calculated in pmoles of incorporated phosphate in the protein acceptor after a 10 minute incubation .

The references are used with appropriate dilutions of DMSO.

The phosphorylation of the substrate can be determined by autoradiography following an SDS-PAGE migration.

The $p34^{cdc2}$/cyclin B is purified from phase M starfish oocytes by affinity chromatography on p9CKShs1-sepharose beads then eluted by use of p9CKShs1 as described above (see (2), (3), and (5)).

For the determination, 1 mg of histone H1 (Sigma type III-S)/ml is used in the presence of 15 mM of [γ ^{32} P]-ATP (3,000 Ci/mmol, 1mCi/ml) in a final volume of 300µl (see (1) and (6)).

After a 10 minute incubation time at 30 deg. Celsius, 25 µl aliquots of supernatant are deposited onto phosphocellulose Whatman P81 paper, and after 20 seconds, the filters are washed five times in a 10 ml phosphoric acid solution per liter of water .

The wet filters are transferred in 6 ml plastic "ampoules de scintillation", then 5 ml of ACS scintillation fluid (Amersham)are added and the radioactivity is measured in a Packard counter.

The kinase activity is expressed in pmol of incorporated phosphate in the Histone H1 for a 10 minute incubation or in percentage of maximal activity.

To perform the kinetics experiments in linear conditions, the end point assay system is used for the $p34^{cdc2}$ but on the basis of preliminary results, appropriate non saturating substrate concentrations are used.

The $p34^{cdc2}$/cyclin B kinase is added so that to obtain a linear activity related to the enzyme concentration and time.

In most cases, this requires a 3 to 10 fold dilution of the enzyme in C buffer.

Velocity data are expressed in pmol incorporated in the substrate per second, and quantity of added enzyme. Apparent inhibition constants are determined by graphical analysis.

- The $p33^{cdk2}$/cyclin A and p 33 cdk2/cyclin E are reconstituted from baculovirus infected sf9 insect cells.

Cyclin A and E are fusion proteins of GST-cyclins and the complexesare purified on glutathione-sepharose beads.

Kinases activities are determined with 1 mg/ml histone H1 (Sigma, type IIIS), in the presence of 15 μM of [γ ^{32} P]-ATP, during 10 minutes, in a 30 μl final volume, as described for the $p34^{cdc2}$/cyclin B.

- The $p33^{cdk5}$ /p25 is purified from bovine brain (7), but the chromatography Mono S step is not used.

The active frations that are recovered from the superose 12 column are pooled and concentrated until a final concentration of about 25 mg of enzyme/ml is reached.

The determination of the kinase is performed with 1mg/ml Histone H1 (Sigma, type III S), in the presence of 15 μM of [γ ^{32} P]-ATP, during 10 minutes, in a 30 μl final volume, as described for the $p34^{cdc2}$/cyclin B.

- The p33 ^{cdk4}/cyclin D1 is obtained from insect cell lysates. Cdk4 is a GST-cdk4 construct, and the active complex is purified on glutathione-sepharose beads.

Its kinase activity is determined with a purified GST-retinoblastome protein in the presence of 15 μM of [γ ^{32} P]-ATP, in a 30 μl final volume.

After a 15 minutes incubation, Laemli buffer is added (2x30 μl).

The phosphorylated substrate is separated by SDS-PAGE 10% and analyzed by autoradiography, by exposure for about 14 hours to MP Hyperfilm and densitometry.

- The p33 ^{cdk6}/cyclin D2 is obtained from insect cell lysates (8). For the assays, the same process, described hereinabove, as with the p33 ^{cdk4}/cyclin D1 is followed.

- The MAP kinases: the GST-erk1 (9), cloned from a human HepG2 library, is expressed in bacteria, purified on glutathione-sepharose beads, and tested with 1 mg of myelin basic protein per ml in the presence of 15 μM of [γ ^{32} P]-ATP, as described hereinabove for the p34^{cdc2}/cyclin B.

  The erk1 and erk2 proteins labeled by the histone, are activated in vitro by MAPKK, purified (affinity-Ni and MonoQ), and tested as described hereinabove during 10 minutes in a 30 μl final volume.

- The catalytic sub-unit of the cAMP-dependent kinase purified from bovine heart is tested with 1 mg histone H1 per ml in the presence of 15 μM of [γ ^{32} P]-ATP, as described hereinabove for the p34^{cdc2}/cyclin B.

- The cGMP-dependent kinase (10) purified from bovine tracheal smooth muscle, is tested with with 1 mg histone H1 per ml in the presence of 15 μM of [γ ^{32} P]-ATP, as described hereinabove for the p34^{cdc2}/cyclin B.

- The casein kinase 2 is isolated from the cytosol of rat liver cells (11) and tested with 1 mg of casein per ml and 15 μM of [γ ^{32} P]-ATP. The substrate is loaded onto Whatman 3MM filters and washed with TCA 10% (W/V).

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- The myosin light chain kinase, purified from hen gizzard (12) is tested in the presence of 100nM calmodulin, 100 μM $CaCl_2$, 50 mM HEPES buffer, 5 mM $MgCl_2$, 1mM DTT and .1 mg BSA/ml pH 7.5, using a peptide synthesized from the phosphorylation site of the smooth muscle myosin light chain (KKRPQRATSNVFAM, 50 μM) and in the presence of 15 μM of [γ ^{32} P]-ATP, in a 50 μl final volume.

  The incorporation of the radioactive phosphate is controlled on phosphocellulose filters as described hereinabove.

- The ASK-γ, plant homologue of GSK-3, is expressed as a GST fusion protein in E. coli (13), and purified on glutathione-sepharose. The activity of the ASK-γ kinase is determined ,

during 10 minutes at 30 deg Celsius, with 5 µg of myelin basic protein, in the presence of 15 µM of [γ^{32} P]-ATP, in a 30 µl final volume. The phosphorylated myelin basic protein is harvested onto Whatman P81 phosphocellulose paper, as previously described for the

$p34^{cdc2}$/cyclin B.

- The tyrosine kinase domain of the Insulin receptor (14) is overexpressed in a baculovirus system and purified until homogeneity. Its kinase activity is determined during 10 minutes at 30deg. Celsius with 5 mg Raytide (Oncogene Sciences), in the presence of 15 µM of [γ^{32} P]-ATP, in a 30 µl final volume. the phosphorylated Raytide product is recovered onto Whatman P81 phosphocellulose paper as described hereinabove for the $p34^{cdc2}$/cyclin B.

Example, 1: Synthesis of roscovitin

The synthesis is achieved in 3 steps and comprises the preparation 1) first of all, of the 6-benzylamino-2-chloropurin, thereafter 2) of the 6-benzylamino-2-chloro-9-isopropylpurin and 3) of the 6-benzylamino-2-R-(1-ethyl, 2-hydroxyethylamino)-9-isopropylpurin.

1) Synthesis of the 6-benzylamino-2-chloropurin:

The same process as described by Hocart in Phytochemistry (1991) 30, 2477-2486

2) Synthesis of the 6-benzylamino-2-chloro-9-isopropylpurin:

A mix of 6-benzylamino-2-chloropurin (3.7 g; 14.2 mmol), potassium carbonate (11g; 80 mmol) and isopropyle bromide (8.2 ml; 87 mmol) in 100 ml of absolute DMSO is subject to agitation at room temperature during three days. Slice chromatography, [CHCl-MeOH (98:2)] of the mix shows that the 6-benzylamino-2-chloropurin is absent. By vacuum-distillation below 50deg Celsius, DMSO and the excess of isopropyl bromide are eliminated. The residue is partitioned between water and ethyl acetate. The organic phase is dried on Na_2SO_4 and vacuum-evaporated.

By crystallization in MeOH, 3.51 g (82%) of product is obtained; F.P.: 181-182 deg Celsius; UV (MeOH): λ_{max} 273.5; I.R. (Nicolet 205, KBr, DRIFT cm 1713, 1626, 1572, 1537, 1497, 1471, 1456, 1425, 1398, 1355, 1314, 1292, 1255, 1228, 1202).

3) Synthesis of the 6-benzylamino-2-R-(1-ethyl-2-hydroxyethylamino)-9-isopropylpurin (II), racemic derivative:

(???)A sealed vacuum phial containing 2.7 g (8.95 mmol) of I and 17 ml (0.18 mol) of R(-)-

2-amino-1-butanol (Fluka 90%, R:S.9:1) is heated in an oven at 160-165 deg Celsius for 3 hours. The excess of amine is evaporated at a temperature below 50 deg Celsius, and the product II is purified on chromatography column by using increasing quantities of MeOH in $CHCl_3$, which means 0, then 2, then 3%.

By crystallization in ethyl acetate, 2.2g of II are yielded (69%); F.P.: 132-134 deg Celsius; $[\alpha]$= +35.1 (c= 0.29, $CHCl_3$). Mass spectrography [Finnigam MAT 90, BE geometry 70 eV, source temperature: 250 deg Celsius, emission current 1 mA, acceleration voltage 5 keV, DIP temperature between 190-220 deg Celsius. HRMS has been performed by the overlapping pikes [using Ultramark 1600 F (PCR Inc.; Fl.; USA) as a standard] 354.2167 (M+, C19H26N6O calc. 354.2168, 27%) 325 (7%), 324 (29%), 232 (100%), 295 (3%), 282 (7%), 281 (3%), 217 (6%), 185 (5%), 134 (3%), 91 (34%). FTIR (Nicolet 205, KBr, DRIFT, cm^{-1}): 1622, 1610, 1547, 1540, 1452, 1389, 1370, 1261, 1068.

Example, 2: Study of the kinases inhibiting properties of roscovitin and the effects thereof on the cell cycle.

a) Study of the kinases inhibiting properties.

The activity of the enzymes reported in the table hereinbelow were measured after addition of increasing concentrations of roscovitin or of olomucin. These activities were measured with appropriate substrates (H1 histone, myelin basic protein, casein, etc...) with 15 μM of ATP.

The IC_{50} were calculated according to the dose-response plots that were obtained. The (--) symbol means that no inhibiting effect was observed. The highest tested concentration is noted into parenthesis.

[see table 2, page 24]

- inhibition of cdc2, cdk2 and cdk5

The results show that the activity of the roscovitin is 10 fold higher than the activity of the olomucin with respect to cdc2 and cdk 2 targets and 20 fold higher with respect to cdk5.

Its effect appears, comparatively, limited as observed with the olomucin (,) on the cdk4/cyclin D1 and cdk6/cyclin D2 (IC_{50} are greater than 100 μM). This absence of effect was confirmed with cdk4 obtained from various sources. In identical conditions, the GST-$p16^{INK4A}$ inhibits the cdk4/cyclin D.

- specificity of the inhibiting effect

Most of the kinases are slightly or not at all inhibited.

Although the roscovitin shows an efficiency at least 10 fold greater than the efficiency of the

olomucin on its cdk targets, its inhibiting effect is very similar to the one shown by the olomucin on erk1 and erk2.

A 40 fold higher concentration of roscovitin is necessary to inhibit erk1 (20 fold for erk2) in a similar way as to inhibit cdc2.

b) effect on ATP:

To study the mechanism of action of the roscovitin, kinetics experiments were performed in the presence of increasing concentrations of roscovitin, with variation of the ATP levels (from 0.1 to 0.5 mM), the concentration in Histone H1 is maintained constant at 0.7 mg/ml.

The results are reported on figure 1.

These results show that the roscovitin acts as a competitive inhibitor for the ATP. Considering that the plots are linear to the concentrations in roscovitin, the inhibitor is called linear inhibitor. The apparent inhibition constant Ki is 1.2 µM.

The analysis of the structure of the roscovitin co-cristal and of cdc2 confirm that the roscovitin binds, like the olomucin, to the ATP binding pocket and that its purin cycle is oriented in the same way as the one of the olomucin, that is to say, in a totally different manner from the orientation of the purin cycle of the ATP.

c) Effect on DNA synthesis and MPF activity:

These are the results of experiments performed on several cell types.

- Effect on the maturation of starfish oocytes and on the dephosphorylation of the[3] Tyrosine of p34 ^{cdc2}, *in vivo*.

Starfish oocytes, stopped in prophase, are treated during 15 minutes, with increasing concentrations of roscovitin, before addition of 1-MeAde hormone (1µM).

After 30 minutes, the percentage of germinal vesicle disruption (GVBD) is noted. These data

[3] In the French text, the use of the definite article "la" suggests that one particular tyrosine residue is the dephosphorylation target vs. part or all of the others contained in the molecule. However, it probably relates to any tyrosine since no number is associated with the residue.

are reported on figure 2, in function of the roscovitin concentration (μM). Roscovitin inhibits the disruption of the nuclear envelope, with an IC_{50} of 5μM. (The IC_{50} of the olomucin, in the same conditions, equals 30μM). These results are reported on figure 2.

As already observed, the roscovitin decreases, but does not inhibit, the

dephosphorylation of the p34 ^{cdc2}, *in vivo*. Oocytes are treated with 10 μM roscovitin during 15 minutes, before addition of 1-MeAde 1μM, at time 0. Extracts are prepared at various times, and loaded onto a column of p9 $^{ckshs-1}$ sepharose beads.

The proteins that bound to the beads are separated by SDS-PAGE, before western-blot detection by anti-PSTAIRE antibodies. A photo of the Western-Blot is represented on figure 3. Phosphorylated forms of the p34 ^{cdc2} appear on the top part, and dephosphorylated forms are located on the lowest part.

The roscovitin, therefore, does not inhibit the activation of the cdc2, but inhibits its activity. The dephosphorylation of the p34 ^{cdc2} tyrosine is catalyzed by cdc25, and normally occurs before the activation of the cdc 2 kinase at the G2/M transition. Moreover, the cdc 2 kinase phosphorylizes and enhances the activity of the cdc25 phosphatase. The roscovitin may therefore have provoked an interruption at the cdc 2 kinase level, resulting in a diminution of the dephosphorilyzation.

- Effects on the mitotic cycle of urshin embryos

Roscovitin is added 60 minutes after fertilization. The percentage of divided embryos is noted 120 minutes after fertilization. The results are reported on figure 4.

Roscovitin is shown to stop mitosis at the late prophase stage, in a dose-dependent manner.

The IC_{50} equals 10μM (even at 100μM, the olomucin only slows down the prophase/metaphase transition, but does not stop the cells in prophase).

A large nucleus is observed in the eggs blocked by roscovitin, as represented on figure 5.

This blocking effect is totally reversible. Indeed, after several washes with sea-water, the eggs enter again the mitotic cycle and evolve into normal pluteus larvae. These results are obtained even at high

concentration in roscovitin, of 100μM).

- Effects on the DNA synthesis in vitro, and on the MPF activity, in Xenopus egg extracts

The assays are performed according to (15), operating as described in (1) for the olomucin.

Xenopus extracts, arrested at the metaphase stage by roscovitin and sperm chromatin, are subjected to incubation.

With roscovitin concentrations from 0 to 5μM, the chromosomes stay strongly condensed, and no nuclear envelope is visible. At a 10 μM concentration, and above, interphase nuclei appear with partially decondensed chromatin, and an intact nuclear envelope, showing that the MPF activity was inhibited. (IC_{50} equals 5μM).

The inhibition of the DNA synthesis was also studied, operating as described in (1) for the olomucin.

Roscovitin and sperm chromatin were added to egg extracts stopped at the metaphase stage.

The extract was then left in interphase by addition of $CaCl_2$ (15) and (16). The synthesis of total DNA was measured 3 hours alter by [γ-^{32}P] dATP incorporation, in TCA precipitable material.

As shown on figure 6, roscovitin inhibits replication with an IC_{50} of 15μM.

The invention then provides new purins that have highly specific cdc2/cyclinB inhibiting properties.

Example 3: Biochemical properties and effects of roscovitin on mammalian cells:

Method

- *in vitro* screening of human tumor cells.

60 human tumor cell lines comprising 9 types of tumors were cultivated for 24 hours, prior to a 48 hour continuous exposure to 0.01-100 µM roscovitin. To assess the cytotoxicity, a sulforhodaminin B protein test was used.

- L1210 cell culture.

L1210 cells were obtained from exponential phase cultures, and cultivated in

RPMI-1640 medium, 10% FCS, penicillin and streptomycin. The cells were counted in an hemocytometer, and cultivated at a 5.10^4cells/ml ratio[4], in 96 wells tissue culture plates, in the presence or absence of various concentrations of roscovitin or olomucin, and thereafter incubated at 37 degrees Celsius under 5% CO_2. To revert the effects of the roscovitin, the L1210 cells, cultivated for 2 days in the presence or absence of roscovitin, were washed in PBS, in order to eliminate all traces of active product, counted and thereafter placed again in fresh active product-free medium (roscovitin or olomucin).

The cell growth was measured every day, using the tetrazolium test on microculture. The analysis of the cell cycle was achieved on ethanol fixed cells with 100µg/ml RNase and dyed by propidium iodide. Data were retrieved from a Coulter Flow cytometer (Hialeah, FL, USA) EPICS Elite (registered trademark) and the analysis of the data was performed using a Multicycle software (Phoenix Flow Systems, San Diego, CA, USA) (registered trademark). All these tests were performed in triplicate and all the experiments were repeated at least twice.

- *In vivo* phosphorylation of vimentin.

To study the *in vivo* phosphorylation of vimentin by the cdc2 kinase, cells were treated or not with 60 µM roscovitin for 48 hours prior to a 10 ng/ml colcemide exposure for an additional 2 hours. The cellular extracts were then loaded onto a SDS-PAGE for migration, transferred by Western-Blot, and incubated with 4A4 antibodies. These antibodies cross-react with cdc2 phosphorylated vimentin, but do not react either with vimentin phosphorylated by other kinases (cAMP-dependent protein kinase, C protein kinase, Ca^{2+} - calmodulin dependent protein kinase) nor with unphosphorylated vimentin. 4A4 antibodies specifically recognize vimentin that is phophorylated at its Ser55 residue by the cdc2 kinase while engaging into the cell mitosis.

Results:

The roscovitin(0.01-100 µM, 48 hour exposure) was tested on 60 human tumor cell lines comprising 9 tumor types (leukaemia, non-small-cell-lung cancer, colon cancer, central nervous system cancer, melanoma, ovarian cancer, kidney cancer, prostate cancer, and breast cancer). All the cell lines displayed

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[4]exact translation, should be "concentration"

an equal sensitivity to roscovitin. The average IC_{50} is 16μM (though it equals 60, 3[5] μM for the olomucin). No correlation was observed between the cell lines sensitivity to roscovitin, and the presence of wild-type or mutated p53. The Compare analytical method showed that these effects of roscovitin and of flavopiridol are comparable.

Regarding the effects of roscovitin on the growth of the L1210 cell line, a very clear dose-dependent inhibition was observed, as shown on figure 7A. On figure 7A, the cell growth is represented in function of the concentration in roscovitin or olomucin. The plots are rather identical, after 2 to 3 day cultures, as observed with the abovementioned tumor cells. The roscovitin is approximately 4 times more efficient than the olomucin to inhibit the cell growth (IC_{50} is 40μM for the roscovitin, and 160μM for the olomucin). Although most of the cells are viable (60± 2% by trypan blue exclusion) after a 48 hour treatment with roscovitin 60 μM, they stay irreversibly blocked, even after extensive washings. Cells exposed to 120 μM roscovitin rapidly die. The effects of roscovitin on the cell cycle distribution were then studied by flow cytometry. At 60 μM roscovitin, cells are arrested in G1 and accumulate in G2, as shown on figure 7B, where the proportions (%) of each cell cycle phase (G1, S, G2/M) in the presence or absence of roscovitin, were represented. In order to identify the *in vivo* molecular target of roscovitin, 4A4 antibodies were used. The results are illustrated on figure 8, where are represented the total proteins extracted from roscovitin-treated (+) or untreated (-) cells then separated on SDS-PAGE, before Western Blotting and detection by 4A4 antibodies[6]. Untreated cells stop in metaphase and display cdc2 phosphorylated vimentin, which demonstrated that cdc2 was actually in vivo inhibited and that the cells were blocked before metaphase.

Roscovitin also contributes to the reduction of the tau protein hyperphosphorylation that is observed in Alzheimer disease: one brain-specific cdk (cdk5/p35) phosphorylizing certain sites of tau, is especially sensitive to roscovitin.

CLAIMS

1- Biologically active purin derivatives characterized in that they have the following formula:

[formula in original]

in which

- R2, R6 and R9, identical or different from one another, represent an halogen atom, a R-NH, R-NH-NH-, NH2-R'-NH or R-NH-R'-NH- radical, in which R represents a straight or branched chain,

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[5] ambiguity in the numbering system-the French system uses coma where tha anglo-saxon system uses the decimal point. Should be: 60.3 mM.

[6] exact translation of the original text. Should be: "Western Transfer with the 4A4 antibodies!

saturated or unsaturated alkyl radical an aryl or cycloalkyl radical or an heterocycle, and R', a straight or branched, saturated or unsaturated akylen group, or an arylen or cycloalkylen group, R and R' containing each 1 to 8 carbon atoms, and potentially subsituted by one or several -OH, halogen, amino or alkyl groups,

- R2 can represent, besides, an heterocycle potentially** bearing a straight or branched , saturated or unsaturated alkyl radical, an aryl or cycloaryl radical, or an heterocycle, potentially** substituted by one or several -OH, halogen, amino, or alkyl groups,

- R9 can represent, besides, a straight or branched , saturated or unsaturated alkyl radical, an aryl or cycloalkyl radical,

- R2 and R9 can represent, besides, a hydrogen atom, except for the derivatives in which the substituants resectively have the following signification:

R6 and R9, benzylamino and methyl groups

R2 and R6, 2-hydroxyethylamino and benzylamino groups

R2, R6, and R9 , an amino, benzylamino and methyl group, or chloro, amino and methyl group, or chloro, benzylamino and methyl group, or chloro, 3-hydroxybenzylamino and methyl group, or chloro, 5-hydroxypentylamino and methyl group, or 2-hydroxyethylamino, benzylamino and isopropyl group, or 2-hydroxyethylamino, amino and methyl group, or 2-hydroxyethylamino, isopentenyl and methyl group, or 2-hydroxyethylamino, isopentenylamino and methyl group, or 2-hydroxyethylamino, benzylamino and methyl group, or 2-hydroxyethylamino, benzylamino and 2-hydroxyethyl group, or 2-hydroxyethylamino, benzylamino and isopropyl group, or 2-hydroxyethylamino, (3-hydroxybenzyl)amino and methyl group, or 2-hydroxyethylamino, (3-hydroxybenzyl)amino and isopropyl group, or 2-hydroxyisobutylamino, 6-benzylamino and methyl group, 2-hydroxyethylamino, isopentenylamino and isopropyl group, or (2-hydroxyethyl)amino, (4-methoxybenzyl)amino and isopropylamino,

and characterized in that their IC_{50} is lower than or equal to about 5 μM for cdc2/cyclinB.

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**potentially: .. "heterocycle potentially bearing ..." equivalent to "...heterocycle that MAY bear..." vs. "...heterocycle , potentially substituted by...", equivalent to "heterocycle that MIGHT potentially be substituted by...". This nuance is beared by the French adverb "eventuellement" which must not be translated into "eventually", and cannot be faithfully translated.

2- Purine derivatives according to claim 1, characterized in that the halogen atom is preferably chosen among Chlorin, Bromine or Fluorine, the alkyl radical, among the methyl, ethyl, propyl, isopropyl, butyl and isobutyl, pentyl, hexyl and heptyl groups;

- the alkylen radical, among the methylen, ethylen, propylen, isopropylen, butylen, isobutylen, penten or isopenten groups;

- the aryl radical is a benzyl group;

- the cycloalkyl is a cyclohexyl;

- the arylen radical is a benzylen group;

- the cycloalkylen radical is a cyclohexylen group;

-the heterocycle is a nitrogen and/or oxygen-heterocycle such as an imidazol, oxadiazol, pyridin, pyridazin or pyrimidin or else, a pyrrolidin.

3- Purine derivatives according to claim 1 or 2, wherein R2 is chosen among the radicals that can bind , in a cdk2/ATP complex, a region of the ATP-binding pocket occupied by the ribose residue[7].

4- Purin derivatives according to claim 3, characterized in that R2 is chosen among a chlorin atom, the amino, methylamino, ethylamino, n-heptylamino, aminoethylamino, aminopropylamino, dimethylaminoethylamino, hydroxyethylamino, hydroxypropylamino, hydroxyisobutylamino, hydroxypentylamino or hydroxymethylpropylamino, [(2R)-2-hydroxymethyl-pyrrolidin-1-yl], N- benzyl-aminoethanol, (R,S)-amino-hexanol, (S)-amino-2-phenylethanol, (R)-amino-2-phenylethanol, (R)-amino-3-phenylpropanol, (R,S)-amino-pentanol, (R)-amino-propanol, (S)-amino-propanol, (R)-N-pyrrolidin methanol groups.

5- Purin derivatives according to claim 4, wherein R2 is a hydroxypropylamino group.

6- Purin derivatives according to any of claims 1 to 5, characterized in that R6 is chosen among the amino, isopentenylamino, hydroxypentylamino, 4-hydroxy-3-methyl-trans-2-butenylamino, benzylamino hydroxybenzylamino, hydroxyethyl benzylamino, cyclohexylmethylamino, isopenten, benzylamino or (3-iodo)-benzylamino groups.

7- Purine derivatives according to claim 6, wherein R6 comprises an hydrophobic residue, such as benzyl or hydroxybenzyl.

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[7] exact translation, but no sense

8- Purine derivatives according to any of claims 1 to 7, wherein R9 is chosen among the hydrogen atom, a methyl, isopropyl or hydroxyethyl radicals.

9- Purine derivatives according to claim 8, wherein R9 is a hydrophobic group such as the isopropyl group.

10- Purin derivatives, characterized in that they are chosen among the 2-chloro-6-(3-hydroxybenzylamino)-9-methylpurine, 2-(hydroxypropylamino)-6-benzylamino-9-isopropylpurine, 2-D,L-(2-hydroxypropylamino)-6-benzylamino-9-isopropylpurine, 2-(5-hydroxypentylamino)-6 benzylamino-9-isopropylpurine, 2-(1-D,L-hydroxymethylpropylamino)-6-benzylamino-9-isopropylpurine, 2-(aminoethylamino)-6-benzylamino-9-isopropylpurine, 2-bis(2-hydroxyethylamino)-6-benzylamino-9-isopropylpurine, 2-(2-hydroxypropylamino)-6-isopentenylamino-9-isopropylpurine, 2-(2-hydroxyethylamino)-6-cyclohexylmethylamino-9-methylpurine, 2-(2-hydroxyethylamino)-6-benzylamino-9-methylpurine, 2-(2-hydroxyethylamino)-6-benzylamino-9-isopropylpurine, 2-chloro-6-isopentenylamino-9-isopropylpurine, 2-(2-hydroxyethylamino-6-(3-hydroxybenzylamino)-9-methylpurine, non cristallin 6-benzylamino-2-[(2R)-2-hydroxymethyl-pyrrolidin-1-yl]-9-isopropyl-(9H)-purine, 2-[6-benzylamino-9-isopropyl-(9H)-purine-2-yl]-N-benzylaminoethanol, 2-(R,S)-[6-benzylamino-9-isopropyl-(9H)-purine-2-yl]-amino-hexanol, 2-(S)-[6-benzylamino-9-isopropyl-(9H)-purine-2-yl]-amino-2-phenylethanol, 2-(R)-[6-benzylamino-9-isopropyl-(9H)-purine-2-yl]-amino-2-phenylethanol, 2-(R)-[6-benzylamino-9-isopropyl-(9H)-purine-2-yl]-amino-3-phenylpropanol, 2-(R,S)-[6-benzylamino-9-isopropyl-(9H)-purine-2-yl]-amino-pentanol, 2-(R)-[6-benzylamino-9-isopropyl-(9H)-purine-2-yl]-amino-propanol, 2-(S)-[benzylamino-9-isopropyl-(9H)-purine-2-yl]-amino-propanol, 2-(R)-(-)-[6-(3-iodo)-benzylamino-9-isopropyl-(9H)-purine-2-yl]-N-pyrrolidin methanol, and 2-(R)-(-)-[6-benzylamino-9-cyclopentyl-(9H)-purine-2-yl]-N-pyrrolidin methanol.

11- Purine derivatives according to claim 10, characterized in that they are chosen among, 2-(3-hydroxypropylamino)-6-benzylamino-9-isopropylpurine, 2-D,L-(2-hydroxypropylamino)-6-benzylamino-9-isopropylpurine, 2-(aminoethylamino)-6-benzylamino-9-isopropylpurine, 2-bis(2-hydroxyethylamino)-6-benzylamino-9-isopropylpurine, 2-(2-hydroxypropylamino)-6-isopentenylamino-9-isopropylpurine, non cristallin 6-benzylamino-2-[(2R)-2-hydroxymethyl-pyrrolidin-1-yl]-9-isopropyl-(9H)-purine, 2-[6-benzylamino-9-isopropyl-(9H)-purin-2-yl]-N-benzylaminoethanol, 2-(R,S)-[6-benzylamino-9-isopropyl-(9H)-purine-2-yl]-amino-hexanol, 2-(S)-[6-benzylamino-9-isopropyl-(9H)-purine-2-yl]-amino-2-phenylethanol, 2-(R)-[6-benzylamino-9-isopropyl-(9H)-purine-2-yl]-amino-2-phenylethanol, 2-(R)-[6-benzylamino-9-isopropyl-(9H)-purine-2-yl]-amino-3-phenylpropanol, 2-(R,S)-[6-benzylamino-9-isopropyl-(9H)-purine-2-yl]-amino-pentanol, 2-(R)-[6-benzylamino-9-isopropyl-(9H)-purine-2-yl]-amino-propanol, 2-(S)-[6-benzylamino-9-isopropyl-(9H)-purine-2-yl]-amino-propanol, 2-(R)-(-)-[6-(3-iodo)-benzylamino-9-isopropyl-(9H)-purine-2-yl]-N-pyrrolidin methanol, and 2-(R)-(-)-[6-benzylamino-9-cyclopentyl-(9H)-purine-2-yl]-N-pyrrolidin methanol.

12- Optical isomers and the racemic mixtures, and geometric isomers, of the derivatives according to any of claims 1 to 11.

13- The 2-(1-D,l-hydroxymethylpropylamino)-6-benzylamino-9-isopropylpurine.

14- Pharmaceutical compositions containing an efficient quantity of at least one of the purin derivatives according to any of claims 1 to 13 in association with a pharmaceutical vehicle.

15- Pharmaceutical compositions according to claim 14, characterized in that they can be administered orally or by injection.

16- Pharmaceutical compositions according to claim 15, characterized in that they are tablets, caplets, capsules, pills, and that they contain 1 to 100 mg of active principle per dose, preferably 10 to 40 mg.

17- Pharmaceutical compositions according to claim 15, wherein the solution for injection advantageously contains, per dose, from 1 to 50 mg of active principle, preferably from 10 to 30 mg.

18- Pharmaceutical compositions according to any of claim 14 to 17, characterized in that they are used as antimitotic drugs, particularly for chemotherapy of cancers, or for the treatment of psoriasis, parasitosis such as those from fungi and protists, or of the Alzheimer disease.

19- Pharmaceutical compositions according to any of claims 14 to 17, characterized in that they are utilized as antineurodegenerative drugs, especially antineuronal apoptosis.

20- Weedkiller compositions, wherein there is at least one purin derivative according to claim 1 to 13, potentially in association with other phytopharmaceutical agents.